IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Tabor et al.
Title:	ISOTHERMAL AMPLIFICATION OF DNA
Appl. No.:	10/813,693
Filing Date:	10/7/2003
Examiner:	Bertagna, A.M.
Art Unit:	1637
Conf. No.:	4141

DECLARATION UNDER 37 CFR § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Stanley Tabor, being duly warned, hereby declare and say that:

1. I have extensive experience as a researcher and scientist in DNA replication, especially with respect to amplification technologies and T7 DNA replication system. I hold the position of Lecturer in Biological Chemistry and Molecular Pharmacology at Harvard University, the assignee of the US Patent Application No. 10/813,693 (the "'693 Application"). I am an inventor or co-inventor on more than 15 issued U.S. patents and more than 100 issued and pending patents worldwide. In addition, I have authored more than 40 scientific publications. I received a B.S. in Biology from Stanford University and a Ph.D. in Biological Chemistry from Harvard University. A copy of my Curriculum vitae is attached hereto as Appendix 1.

2. I am an inventor of the above identified patent application.

3. I have read and am familiar with the Office Action dated December 11, 2007; the response and amendment filed May 12, 2008; and the Office Action dated October 1, 2008. In particular, I have read and am familiar with the amended claims filed with the May 12, 2008 response; and I have reviewed the cited references of Scherzinger et al. (European Journal of Biochemistry (1977) 72: 543-558), Sorge et al. (US 5,556,772), and Tabor et al. (Journal of Biological Chemistry (1989), 264(11): 6447-6458).

4. The claims of the '693 application as submitted with the May 12, 2008 response are directed to methods of amplifying a template DNA molecule that involve incubating the template DNA molecule with a reaction mixture including a DNA polymerase and at least one accessory protein at a constant temperature to produce an amplified DNA product. The amplification methods do not require exogenously-added oligonucleotide primers and the template DNA molecule does not have a terminal protein covalently bound to either 5'-end. The methods result in an amplified product that is in an amount of at least 10-fold greater than the amount of template DNA put into the mixture.

5. The claims are based at least in part on the surprising results of the experiments which demonstrate that template DNA molecules could be amplified well over 10-fold. Amplification of template DNA can be as much as 10,000,000-fold or more, without the need for exogenously added primers and without the need for a terminal protein covalently bound to either 5'-end under isothermal conditions. See, for example, '693 application at page 5, line 23 through page 6, line 27; page 7 lines 8-15; page 8 lines 3-15, Examples 1, 2, and 4. For example, using a reaction mixture described in Example 1 of the '693 application, double stranded plasmid DNA was amplified 150,000-fold. Prior to these findings first reported in the '693 application, there was nothing in the scientific literature or in the field in general suggesting that a 10-fold or greater amplification of DNA could possibly be achieved under the above conditions, much less a 10,000,000-fold or more amplification.

6. I understand that the Examiner has rejected the claims as allegedly being unpatentable in view of several references that include Scherzinger et al., Sorge et al., and Tabor et al.

7. Scherzinger et al. discloses that T7 DNA-priming protein is capable of synthesizing RNA primers which are utilized by T7 DNA polymerase in DNA replication to achieve an amplification of at most 4-fold under isothermal conditions (Scherzinger et al. at p.549, col. 2). To achieve greater than 10-fold amplification of the input target DNA would require modifying Scherzinger's method in a way that increases the performance by at least a factor of 2.5 or more. Furthermore, to achieve the claimed 10,000,000-fold amplification would require modifying Scherzinger's method in a way that would increase the amplification by a factor of more than 2,500,000. Scherzinger et al. provides no guidance as to how one might do so and such improvements necessarily go beyond the routine optimization of assay conditions. Those working in the field before October 2003 may have expected that optimizing the conditions of the Scherzinger reactions could result in a modest increase in the DNA amplification, however, there would have been no reasonable expectation that optimizing the methods described by Scherzinger et al. could result in a 10-fold amplification of DNA, much less a 10,000,000-fold and exponential amplification as claimed.

8. Sorge et al. discloses a method of DNA amplification that requires exogenously added oligonucleotide primers and T7 DNA polymerase. The method of Sorge et al. is very different from that of Scherzinger et al. Sorge's method requires the use of exogenous primers in the DNA amplification whereas Scherzinger's method does not require exogenous primers. Scherzinger's method requires a T7 DNA-priming protein capable of synthesizing RNA primers which are subsequently utilized by T7 DNA polymerase for DNA replication. There was no guidance in these references to logically combine the disparate methods of Scherzinger and Sorge.

9. Tabor et al. discloses that 3'-5' exonuclease activity of T7 DNA polymerase can be reduced by site-directed mutagenesis. Tabor et al. does not disclose methods of amplifying a template DNA molecule that involve incubating the template DNA molecule with a reaction mixture including a DNA polymerase and at least one accessory protein at a constant temperature that is free from exogenously-added oligonucleotide primers and a terminal protein covalently bound to either of the 5'-ends of the template DNA molecule such that the amount of amplified DNA product is at least 10-fold greater than the amount of template DNA put into the mixture.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Respectfully submitted,

Date February 27, 2009

Stanley Tabor

Stanley Tabor

BIOGRAPHICAL SKETCH

NAME	TITLE	BIRTHDATE
Stanley Tabor	Lecturer in Biological Chemistry and Molecular Pharmacology	January 18, 1954

EDUCATION

INSTITUTION AND LOCATION	DEGREE CONFERRED	YEAR	FIELD OF STUDY
Stanford University	B.S.	1977	Biology
Stanford University	M.S.	1977	Biology
Harvard University	Ph.D.	1987	Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

Research Experience

1977-1987	Graduate student in the Department of Biological Chemistry, Harvard Medical School. Thesis supervisor: Charles C. Richardson. cloning, overexpression, purification, and characterization of proteins of bacteriophage T7 DNA replication.
1987-1989	Research Fellow in Biological Chemistry and Molecular Pharmacology, Harvard Medical School. Laboratory of Charles C. Richardson. Characterization of the DNA polymerase of phage T7.
1989-1990	Senior Research Fellow in Biological Chemistry and Molecular Pharmacology, Harvard Medical School.
1990-	Lecturer of Biological Chemistry and Molecular Pharmacology, Harvard Medical School.

Advisory Boards

1978-1980	Member, Committee on Recombinant DNA Research, Harvard Medical School
1986-1989	Member, Committee on Animal Welfare, Genetics Institute

1984-present Member, Scientific Advisory Board, United States Biochemical Corp.
(in 1993 became a part of Amersham Life Science, Inc which
in 1997 became a part of Amersham-Pharmacia-Nycomed, Inc.)

PUBLICATIONS

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3. Tabor, S., and Richardson, C. C. (1981) Template recognition sequence for RNA primer synthesis by the gene 4 protein of phage T7. *Proc. Natl. Acad. Sci., U.S.A.* **78**, 205-209.
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6. Tabor, S., and Richardson, C. C. (1991) Use of processive DNA polymerases and T7 DNA polymerase with low exonuclease activity for DNA sequence analysis. U. S. patent number 4,994,372.

7. Tabor, S., and Richardson, C. C. (1992) An apparatus for sequencing DNA based on the relative intensity of adjacent bands. U. S. patent number 5,122,345.

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